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## ELEVATION OF ARTERIAL PRESSURE IN RATS BY TWO NEW VERTEBRATE PEPTIDES: FLFQPQRF-NH<sub>2</sub> AND AGEGLSPFWSLAAPQRF-NH<sub>2</sub> WHICH ARE IMMUNOREACTIVE TO FMRF-NH<sub>2</sub> ANTISERUM

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### ABSTRACT

We found that two recently characterized neuropeptides Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH<sub>2</sub> (F-8-F-NH<sub>2</sub>) and Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH<sub>2</sub> (A-18-F-NH<sub>2</sub>) elevate mean arterial blood pressure (MAP) in conscious, unrestrained rats. The pressor activities of both agents were attenuated, but not abolished, by prior treatment with guanethidine or prazosin. These results suggest that F-8-F-NH<sub>2</sub> and A-18-F-NH<sub>2</sub> elevate MAP in rats by potentiating the release of catecholamines and by mechanisms independent of catecholamine release.

### INTRODUCTION

The cardioexcitatory peptide, Phe-Met-Arg-Phe-NH<sub>2</sub> (FMRF-NH<sub>2</sub>) was first isolated and characterized by Price and Greenberg from ganglia of the clam *Macrocallista nimbosa* (1). Subsequently, FMRF-NH<sub>2</sub>-like peptides which are structurally distinct from FMRF-NH<sub>2</sub> were detected in CNS neurons of many mammalian species using antiserum raised against FMRF-NH<sub>2</sub> (2, 7, 9, 10). Recently, three of these FMRF-NH<sub>2</sub>-like peptides were isolated and chemically characterized to be Leu-Pro-Leu-Arg-Phe-NH<sub>2</sub> in chicken brain (3); Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH<sub>2</sub> (F-8-F-NH<sub>2</sub>) and Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH<sub>2</sub> (A-18-F-NH<sub>2</sub>) in bovine brain (11). F-8-F-NH<sub>2</sub> and A-18-F-NH<sub>2</sub> were found to be unevenly distributed in CNS with the highest concentrations in dorsal spinal cord, periaqueductal grey and medulla pons and the lowest concentrations in cortex, striatum and cerebellum (5). Although intraventricularly injected F-8-F-NH<sub>2</sub> was found to attenuate morphine induced analgesia (11), the physiological role of F-8-F-NH<sub>2</sub> and A-18-F-NH<sub>2</sub> still remains to be ascertained. Both FMRF-NH<sub>2</sub> and Leu-Pro-Leu-Arg-Phe-

We here report that both A-18-F-NH<sub>2</sub> and F-8-F-NH<sub>2</sub> have substantial ability to elevate mean arterial blood pressure (MAP) in conscious, unrestrained rats. Furthermore, the ability of both neuropeptides to elevate MAP is partially, but not completely, attenuated by prior treatment with guanethidine or prazosin. The results suggest that both neuropeptides may have roles in the regulation and maintenance of blood pressure by release of catecholamines as well as actions that do not require catecholamine release.

**Materials** Male Sprague-Dawley rats (200-300 g) were obtained from Taconic Farms, MD. A-16-F-NH<sub>2</sub> and F-8-F-NH<sub>2</sub> were custom synthesized by Peninsula Laboratories, Inc. (Belmont, CA) and purified by reverse-phase high pressure liquid chromatography. Prazosin was a gift of Darwin Cheney, (Ciba-Geigy); guanethidine was from Ciba-Geigy.

**Dose Response Studies** Peptides were dissolved in saline and injected via external jugular catheters with MAP and HR being continuously recorded. Each dose (0.1-0.4 ml) was followed by an 0.3 ml flush of sterile saline. For pre-treatment studies, prazosin (100 µg/kg iv via slow infusion) or guanethidine (15 mg/kg; s.c.) were given and single dose studies (100 µg/kg each of F-8-F-NH<sub>2</sub> and A-18-F-NH<sub>2</sub>) performed after the MAP had stabilized.



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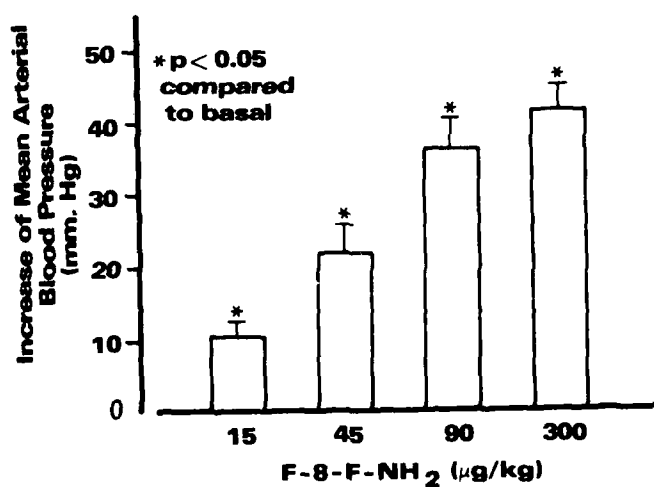
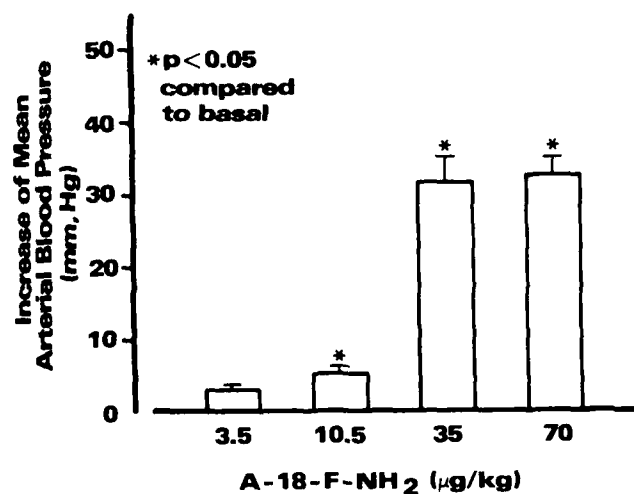


Figure 2. Dose-Response to A-18-F-NH<sub>2</sub> and F-8-F-NH<sub>2</sub> in Conscious Rats. Dose response studies were performed for A-18-F-NH<sub>2</sub> (Top) and F-8-F-NH<sub>2</sub> (Bottom) on MAP in conscious, unrestrained rats. Data represent mean  $\pm$  SEM of 4-6 separate determination. (\*p 0.05 vs saline infusion; t-test). The mean arterial pressure of saline treated rats was  $114 \pm 3.4$  mm Hg; this basal level was set as 0.

## RESULTS

A-18-F-NH<sub>2</sub> and F-8-F-NH<sub>2</sub> elevate MAP. Fig. 1 shows a typical response to injection of 100 µg/kg (92.3 nmole/kg) F-8-F-NH<sub>2</sub>. As is seen, the effect of A-18-F-NH<sub>2</sub> was not immediate and was sustained for 30-60 sec. Dose response studies for A-18-F-NH<sub>2</sub> and F-8-F-NH<sub>2</sub> revealed that both neuropeptides caused an approximate 40 mm Hg increase in MAP, but that considerably higher doses of F-8-F-NH<sub>2</sub> were required both on a molar as well as a µg/kg basis (Fig. 2). A threshold dose of 10.5 µg/kg (5.5 nmole/kg) was obtained for A-18-F-NH<sub>2</sub> while F-8-F-NH<sub>2</sub> required 15 µg/kg (13.9 nmole/kg). Maximal elevations of MAP were obtained with 35 µg/kg A-18-F-NH<sub>2</sub> (18.2 nmole/kg) and 300 µg/kg F-8-F-NH<sub>2</sub> (277 nmole/kg).

TABLE I

Effects of Prazosin and Guanethidine Pre-treatment  
on the Pressor Effects of A-18-F-NH<sub>2</sub> and F-8-F-NH<sub>2</sub>

Peptide	Percent Maximal Response	
	Prazosin	Guanethidine
A-18-F-NH <sub>2</sub> (100 µg/kg)	29.2 ± 3.4*	45.2 ± 1.4*
F-8-F-NH <sub>2</sub> (100 µg/kg)	44.0 ± 5.8*	65.7 ± 5.53*

\*p 0.05 vs control (pre-treatment response).

Effects of Prazosin and Guanethidine Table I shows that prazosin inhibited by 71% the rise in MAP due to a maximum dose of A-18-F-NH<sub>2</sub> and inhibited by 56% the rise in MAP due to F-8-F-NH<sub>2</sub>.

Table I also shows the effect of guanethidine pre-treatment on the pressor response to both peptides. As was seen with prazosin, guanethidine pre-treatment more effectively inhibited the ability of A-18-F-NH<sub>2</sub> to raise MAP and had lesser effects on F-8-F-NH<sub>2</sub>. In both cases guanethidine pre-treatment did not abolish the effects of A-18-F-NH<sub>2</sub> or F-8-F-NH<sub>2</sub> on MAP.

## DISCUSSION

It is now well known that in mammalian CNS, there is a group of peptides which is immunoreactive to antiserum raised against the molluscan cardioexcitatory neuropeptide FMRF-NH<sub>2</sub>. Recently, two of such peptides, octapeptide (F-8-F-NH<sub>2</sub>)

and octadecapeptide (A-18-F-NH<sub>2</sub>), were isolated from bovine brain and chemically characterized (11). These two peptides are not N-terminally extended forms of FMRF-NH<sub>2</sub> and their biological activities remain to be established.

In the present study, F-8-F-NH<sub>2</sub> and A-18-F-NH<sub>2</sub> were found to elevate mean arterial pressure in conscious, unrestrained rats. Previously, using anesthetized rats, FMRF-NH<sub>2</sub> and LPLRF-NH<sub>2</sub> (a FMRF-NH<sub>2</sub> like peptide of chicken brain) were found to evoke pressor responses when injected i.v. or intracisternally (1). FMRF-NH<sub>2</sub>, LPLRF-NH<sub>2</sub>, F-8-F-NH<sub>2</sub> and A-18-F-NH<sub>2</sub> are structurally quite different, however, they all have the same Arg-Phe-NH<sub>2</sub> at their C-termini. Although the structure-activity relationship of the peptide is not studied here, Arg-Phe-NH<sub>2</sub> may be the molecular feature responsible for the vasopressor responses observed for FMRF-NH<sub>2</sub>, LPLRF-NH<sub>2</sub>, F-8-F-NH<sub>2</sub> and A-18-F-NH<sub>2</sub>. In fact, using FMRF-NH<sub>2</sub> analogues on anesthetized rats, it was found that carboxy terminal Arg-Phe configuration was essential for the pressor activity (6).

The pressor effect of FMRF-NH<sub>2</sub> and LPLRF-NH<sub>2</sub> in the anesthetized rat was suggested to be mediated by release of catecholamines and stimulation of  $\alpha$ -adrenergic receptors because the effect of these two peptides was nearly completely blocked by guanethidine and phentolamine (1). In contrast, the pressor effect of F-8-F-NH<sub>2</sub> and A-18-F-NH<sub>2</sub> in the conscious rat was attenuated but not completely blocked by guanethidine or prazosin. Thus, although a portion of activity of F-8-F-NH<sub>2</sub> and A-18-F-NH<sub>2</sub> may be related to release of catecholamines, these two peptides may have actions independent of catecholamine release as well. However, it should be noted that FMRF-NH<sub>2</sub> and LPLRF-NH<sub>2</sub> were tested in anesthetized rats (1) while F-8-F-NH<sub>2</sub> and A-18-F-NH<sub>2</sub> were tested in conscious rats in this study.

These studies indicate that two neuropeptides, A-18-F-NH<sub>2</sub> and F-8-F-NH<sub>2</sub>, can elevate MAP in conscious rats; whether these two peptides have physiological roles related to the regulation and maintenance of blood pressure remains to be established.

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The experiments reported herein were conducted according to the principles set forth in the guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, DHEW, publication No. (NIH) 85.23.

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